Remarks

Restriction Requirement

To the extent the examiner's requirement reads as a restriction requirement of claims 1-9, the examiner's position is improper under 35 U.S.C. 121. A generic claim - such as claim 1 - cannot be restricted into groups, although the examiner can require an election of species. Applicants could cancel claims 2-9 and claim 1 would still encompass of the subject matter of all of claims 1-9 and be of the same scope. Therefore it is a generic claim. Claim 1 is drawn to a method of making a matrix comprising matrix-enhancing molecules. It is not limited to TGF-beta. All of claims 1-9 are drawn to the same invention as claim 1. To the extent the examiner meant to require an election of species, applicants have elected TGF-beta as the species. Indeed, the art cited by the examiner is not limited to a particular species, as discussed below. Accordingly, once the species of claims 1, 2 and 6-9 has been determined to be allowable over the prior art, the examiner must examine the species of claims 3-5.

The composition claims and method of use claims, 10-23, have been cancelled and will be pursued in divisional applications.

Rejections Under 35 U.S.C. § 102 or 103

Claims 1-2 and 6-9 were rejected under 35 U.S.C. § 102 as disclosed by, or under 103(a) as obvious over WO 94/23740 (Celtrix Pharmaceuticals, Inc.), in view of Dinbergs, et al. (*J. Biol. Chem.* 271(47): 29822-29829, 1996). It is assumed that the examiner meant to include claims 3-5 in this rejection. The rejections are traversed.

The following is a discussion of why the claims are not disclosed by the prior art. It is believed that there is an error in the office action at page 5, since a rejection is made under 35

U.S.C. 102(b). This rejection is improper, since a rejection under 102(b) requires all claimed elements to be present in a SINGLE reference and the examiner has acknowledged that no single reference discloses all claimed limitations (see page 3, last paragraph to page 4, first four paragraphs). This rejection is therefore treated as a rejection under 35 U.S.C. 103.

The Claimed Invention

The claimed invention is a method for making a tissue engineering scaffold for inducing formation of extracellular matrix by cells bound to the scaffold comprising coupling matrix-enhancing molecules to the scaffold in an effective density to elicit production of extracellular matrix without increasing cellular proliferation, wherein when the matrix-enhancing molecules are TGF-β, the TGF-β is coupled to the matrix by a polymer tether having a molecular weight between 2000 and 6000 and is in a density between 1 and 100 ng TGF-β/ml or in a concentration of between about 4×10^{-6} and 4×10^{-3} nmol/ml.

Claim limitations include:

(1) coupling matrix-enhancing molecules to the scaffold

(2) in an effective density to elicit production of extracellular matrix without increasing cellular proliferation,

(3) wherein when the matrix-enhancing molecules are TGF-β, the TGF-β is coupled to the matrix by a polymer tether having a molecular weight between 2000 and 6000 and is in a density between 1 and 100 ng TGF-β/ml or in a concentration of between about 4×10^{-6} and 4×10^{-3} nmol/ml.

The purpose of the invention is to increase production of extracellular matrix (i.e., the acellular material produced by cells such as chondrocytes), without increasing the number of cells. This feature alone differentiates the prior art which only recognized the need to increase the number of cells, not the material produced by the cells. Increased cellular proliferation can be detrimental, since this can lead to scarring and inflammation.

WO 94/23740 (Celtrix Pharmaceuticals, Inc.)

WO 94/23740 discloses a method for stimulating bone formation in an animal by administering to the animal an effective amount of a hydrophilic polymer-conjugated growth factor. WO 94/23740 teaches a method for covalently coupling various growth factors such as TGF-β or TGF-β_2 to a polymer such as polyethylene glycol using a linking compound such as n-hydroxysuccinimide (see page 11, lines 13-28). WO 94/23740 teaches that polymer-conjugated growth factors can stimulate bone formation at lower dose levels as compared to the unmodified growth factor, and at higher dose levels, the polymer-conjugated growth factors promote a net increase in bone formation as compared to the unmodified growth factor which causes a net decrease in bone mass (see abstract).

Bone formation requires cellular proliferation. There is no teaching of increasing the amount of extraceullar matrix.

In summary, WO 94/23740 teaches that growth factors coupled to polymer cause a greater increase in cellular proliferation as compared to the growth factors in solution.

The claims are explicitly drawn to a different method. The claims are drawn to a method wherein (1) growth factor is coupled to a polymeric scaffold (note - not a polymer in solution, which is sufficient to demonstrate that WO 94/23740 is not a reference under 35 U.S.C. 102) and (2) the density of the growth factor does NOT cause an increase in cellular proliferation.

Accordingly, WO94/23740 not only fails to teach the claimed elements but teaches away from them.

Dinbergs, et al. J. Biol. Chem. 271(47): 29822-29829 (1996).

Dinbergs, et al. teach a method for making alginate/heparin-Sepharose microspheres containing various growth factors such as bFGF or TGF-β for purposes of studying the response of smooth muscle cells and endothelial cells to sustained-release delivery of bFGF or TGF-β. (see abstract). Dinbergs, et al. demonstrate that **cellular proliferation** is increased by continuous delivery of TGF-beta in solution compared to a bolus administration (see abstract, Figure 3A, Figure 3B, in particular). Dinbergs, et al. does NOT teach a method for making alginate/heparin-sepharose microspheres having tethered on their surfaces TGF-β as claimed. Growth factor concentrations in the range of 1-10 ng/ml were only used in an investigation of the rate of controlled release of soluble bFGF and TGF-β1 from the extracellular matrix (see *Extracellular Matrix Incorporation and Release of Growth Factors,* page 29823, column 2, last full paragraph, bridging page 29824).

Figure 2B shows the effects of controlled release of bFGF on smooth cell. Figure 3B illustrates the effect of controlled release of TGF-β on smooth muscle cell count as a function of time, *and clearly demonstrated increased cell proliferation.* Although the controlled release of TGF-β does inhibit smooth muscle cell growth as compared to the control sample with no TGF-β, ***there is still a notable increase in cell proliferation.*** The same trend may be seen in Figure 3A, with regard to endothelial cell proliferation.

In summary, Dinsbergs, et al. teaches a method for increasing cellular proliferation. Dinsbergs does not teach binding growth factor to a scaffold as claimed, and also teaches away from a method which does not cause an increase in cellular proliferation.

The combination of WO 94/23740 with Dinbergs, et al.

Neither cited reference discloses any method of increasing extracellular matrix without increasing cellular proliferation.

Neither cited reference discloses binding growth factors to a scaffold for formation of extracellular matrix.

Both references teach away from the claimed method by teaching the use of soluble growth factors (or encapsulated growth factors which are released as soluble growth factors), rather than growth factors immobilized on a scaffold.

There is no motivation to modify the references as applicants have done, nor is there any reason to believe one could use growth factors on a scaffold to increase density of extracellular matrix in the absence of an increase in cellular proliferation. Therefore the references cited by the examiner do not make obvious the claimed method.

The teachings of WO 94/23740 in combination with Dinbergs, et al. do not make obvious the enhanced production of extracellular matrix *without increasing cellular proliferation*. The results of the claimed method are **unexpected** in view of Dinbergs, et al. since Dinbergs leads one skilled in the art to expect cellular proliferation, not increased extracellular matrix production in the absence of cellular proliferation and therefore are not obvious to one with ordinary skill in the art [MPEP 716.02].

Rejection Under 35 U.S.C. § 102

Claims 1-2 and 6-9 were rejected under 35 U.S.C. § 102(b) as being unpatentable over WO 96/27657, in view of Dinbergs, et al. (*J. Biol. Chem.* 271(47): 29822-29829, 1996). This rejection is improper.

Claims may be rejected under 35 U.S.C. § 102 as lacking novelty over a *single* publication which discloses all elements of the claim. As stated in the MPEP, "The distinction between rejections based on 35 U.S.C. 102 and those based on 35 U.S.C. 103 should be kept in mind. Under the former, the claim is anticipated by the reference. *No question of obviousness is present.* In other words, for anticipation under 35 U.S.C. 102, *the reference must teach every aspect of the claimed invention* either explicitly or impliedly. Any feature not directly taught must be inherently present. Whereas, in a rejection based on 35 U.S.C. 103, the reference teachings must somehow be modified in order to meet the claims [MPEP 706.02]." At best, the claims could be rejected under 35 U.S.C. § 103(a) as being obvious over WO 96/27657 in view of Dinbergs, et al. Applicants further assert that a 35 U.S.C. § 103(a) rejection would also be unfounded.

WO 96/27657 (Massachusetts Institute of Technology)

WO 96/27657 discloses cell growth substrates with tethered cell growth effector molecules. The WO 96/27657 publication teaches a method for making a tissue engineering scaffold comprising coupling of various matrix-enhancing molecules to a polymeric matrix via flexible tethers ***for purposes of stimulating proliferation of eukaryotic cells*** (see abstract). WO 96/27657 teaches that localized growth factors result in a higher rate of cell growth and are effective at a lower dosage as compared to soluble growth factor (see page 5, line 27).

The combination of WO 96/27657with Dinbergs, et al.

There is no suggestion in either reference to incorporate the teachings of the other reference. Claims 1-32 of WO 96/27657 are directed to methods of promoting cell proliferation via immobilized growth factors. Dinbergs, et al. show that TGF-β enhancing cellular proliferation (see Figure 3). No reference teaches enhancing deposition of extracellular matrix while not increasing cellular proliferation.

Therefore, even if the teachings of the references are combined, the combination does not suggest the claimed methods because it does not suggest attaching growth factors to a polymeric matrix via tethers in a concentration effective to enhance extracellular matrix formation *without an increase in cellular proliferation.* The examiner incorrectly argues that the WO 96/27657 publication teaches a method of enhancing production of extracellular matrix proteins such as collagen, citing page 17, lines 1-4. This section of the publication is directed to materials for construction of tissue regeneration devices, listing collagen as a candidate since it is a natural polymer. The WO 96/27657 publication teaches a method for increasing **cell growth** and nowhere suggests the potential benefit of enhancing extracellular matrix production **without** increasing cellular proliferation. Dinbergs, et al. fail to demonstrate a method of sustained release of TGF-β without increasing cellular proliferation. The teachings of WO 96/27657 in combination with Dinbergs, et al. do not make obvious the enhanced production of extracellular matrix *without increasing cellular proliferation.* The results of the claimed method are **unexpected** in view of Dinbergs, et al. and therefore not obvious to one with ordinary skill in the art [MPEP 716.02].

Sufficient Proof of Unexpected Results

An example of evidence which proved that the claimed method produced unexpected results is described in *In re Orfeo*, 440 F.3d 439 (C.C.P.A. 1971). In *Orfeo*, the court held that the applicant demonstrated that the claimed azeotropic mixture demonstrated unexpected results and was therefore patentable. This particular mixture used less power during a refrigeration process than was predicted based on the prior art. Applicants used Pennington's law to calculate the power requirements for the claimed azeotrope to a number of mixtures of CHF_3 and $CClF_3$. In each case, the azeotrope had a higher power requirement. However, when the actual power requirement was tested, the azeotrope had a lower power requirement (1.59 HP/ton) than would have been predicted using Pennington's law (1.72 HP/ton). Further, the power requirement for a refrigerant typically increases as a higher pressure refrigerant is employed. However, the claimed azeotrope had a higher pressure than either of its components, but had a lower power requirement than the components (see *Id.* At 440). The court held that this was sufficient proof of unexpected results (*Id.*).

The claimed methods have unexpected results in view of Dinbergs, et al.

Applicants' combination of immobilized growth factor technology and the inhibitory properties of TGF-β result in an unexpectedly improved method of enhancing extracellular matrix formation without an increase in cell proliferation. In many tissue engineering applications it is important to avoid undesirable enhancement of cell growth. For example, in

vascular tissue engineering, over-proliferation of the smooth muscle cells can lead to a failure of the tissue engineering construct due to lumenal narrowing.

Researchers have investigated attachment of growth factors such as TGF to a tissue engineering matrix via a polymeric tether such as polyethylene glycol (see PCT/US96/02851 to Massachusetts Institute of Technology, for example). There are also a number of references teaching that TGF-β can be bound to or dispersed within a synthetic or natural polymeric carrier for controlled release of active growth factor (see Schroeder-Teft, et al., *J. Control. Rel.* 49(2-3): 291-298, 1997; Nicoll, et al., *Cells Materials* 5(3): 231-244, 1995; EP 0 428 541 to Collagen Corporation; U.S. Patent No. 6,013,853 to Athanasiou, et al.). Additional references relate to the use of TGF-b in tissue engineering scaffolds to enhance cell or tissue growth or proliferation, particularly of bone (see EP 0 616 814 to Bristol-Myers Squibb Company, for example). However, none of these references disclose how one can achieve enhanced production of extracellular matrix while not increasing cellular proliferation.

Applicants present data (see Tables 5 and 6) illustrating an increase in extracellular matrix formation without a corresponding increase in cellular proliferation. This result is unexpected in view of the prior art and therefore not obvious to one with ordinary skill in the art.

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RESPONSE TO OFFICE ACTION

Allowance of claims 1-2 and 6-9 is respectfully solicited.

Respectfully submitted,

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Certificate of Facsimile Transmission

I hereby certify that this Response to Office Action, and any documents referred to as attached therein are being facsimile transmitted on the date shown below, to the Commissioner for Patents, U.S. Patent and Trademark Office, Washington, DC 20231.

Patrea Pabst

Date: April 3, 2003

Clean Copy of Claims

Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

1. A method for making a tissue engineering scaffold for inducing formation of extracellular matrix by cells bound to the scaffold comprising coupling matrix-enhancing molecules to the scaffold in an effective density to elicit production of extracellular matrix without increasing cellular proliferation, wherein when the matrix-enhancing molecules are TGF-β, the TGF-β is coupled to the matrix by a polymer tether having a molecular weight between 2000 and 6000 and is in a density between 1 and 100 ng TGF-β/ml or in a concentration of between about 4×10^{-6} and 4×10^{-3} nmol/ml.

2. The method of claim 1 further comprising attaching cells to the scaffold.

3. The method of claim 1 wherein the matrix-enhancing molecules are angiotensin II.

4. The method of claim 1 wherein the matrix-enhancing molecules are insulin-like growth factor.

5. The method of claim 1 wherein the matrix-enhancing molecules are ascorbic acid.

6. The method of claim 1 wherein the matrix-enhancing molecules are covalently coupled to tethers which are covalently coupled to the scaffold.

7. The method of claim 1 wherein the scaffold is a hydrogel.

8. The method of claim 7 wherein the hydrogel is formed of a polymer selected from the group consisting of alginate, collagen, hyaluronic acid, and polyethylene glycol polymers.

9. The method of claim 7 wherein the matrix-enhancing molecules are TGF-β coupled to the hydrogel in a concentration of between about 4×10^{-6} and 4×10^{-3} nmol/ml.

10-23. (cancelled)

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